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14. ABSTRACT

Nanotechnology has the potential to develop silicon-based arrays for sensing biomarkers associated with breast cancer.. Until recently, breast cancer research has focused on a small number of genes or proteins as primary biomarkers. In order to develop patient-specific therapy, tailored for each individual, parallel detection of a large number (~103-104) biomarkers may be required. The experience of the semiconductor industry in developing large scale integrated circuits at very lost cost can lead to similar breakthroughs in array sensors for biomolecules of interest to the breast cancer community. Nanotechnology can meet the need for high throughput, sensitive methods for rapidly recording biomarker profiles of tumors in individual patients. We report results on the development of arrays of conductance sensors of bio-functionalized silicon nanowires. For nanoscale wires, such as those used in this study, the change is primarily due to the contribution of surface states to the conductance. The fractional change is greatest for the smallest sensors, due to the increased surface-to-volume ratio. The fabrication of arrays of conductance based sensors has now been done, and the nanosensors have been characterized using model systems. The utility of these newly fabricated sensors to actual clinical breast cancer practice now remains the main goal of our project.

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INTRODUCTION

This is the annual report that describes the results of a research effort into the development of nanosensors for breast cancer biomarkers**.**

THE PROPOSAL HAD THREE SPECIFIC AIMS:

1. Design of a cantilever based sensor for biomolecular recognition, using Finite-Element simulation.
2. Fabrication of Biofunctionalized Nanoscale Sensors capable of detecting targeted molecules at a concentration of less than 1 ng/ml
3. Characterization of Functionalized nanosensors for selected breast cancer markers, and comparing with existing immunohistochemical and Fluorescence *in situ* hybridization (FISH) techniques on well established biomarkers such as Her-2/*neu*, estrogen and progesterone hormone receptors in tumor tissue, and selected mucin antigens in blood.

We are pleased to report that we have achieved two of the specific aims, and are on the way to completing the third specific aim. Earlier year, we had reported the discovery of a very exciting gating principle using electrical methods that has the potential for significantly enhancing the sensitivity and control of the nanoscale structures. This gating idea has been confirmed, and a paper has been published. The proof-of-concept was performed on sensing pH. We have now shown that the gated nanosensors are sensitive to a breast cancer biomarker and can lead to cheaper and faster methods of detecting biomolecular markers. Studies with samples from patients are being awaited, and we have requested a no-cost extension in order to complete studies from patients.

Key Research Accomplishments

We list here the accomplishments so far:

Task 1: We have used simulations to characterize and develop nanoscale cantilever sensors. Simulations conducted in the presence of water show that hydrodynamic effects have to be taken into account in order to properly characterize the sensitivity of these sensors. At the same time, we have performed electrical conductance measurements on nanonwires in order to assess the sensitivity.

Task 2: We have successfully used electron beam lithography to fabricate nanosensors and have functionalized them using silanization protocols. Some of the nanomechanical structures our collaboration has developed is among the smallest and highest frequency nanomechanical resonator ever constructed. In performing conductance measurements on the structures, we have discovered a very interesting gating effect that can be used to enhance the sensitivity of the nanosensors.

Task 3: **We have successfully shown that the nanosensors respond with sensitivity and specificity to the breast cancer biomarker CA15.3.** A new, more sensitive operating regime for the semiconductor sensors has been discovered.

Reportable Outcomes

We have used simulations to characterize and develop several types of nanosensors. Figure 1 shows an electron microscope image of a single silicon nanowire with contacts on either end. The first device fabricated shown has no control gates or additional electrodes, and was used to test functionalization protocols. The nanowire conductance is modulated as selected biomarker targets bind to the surface of the nanowire. Figure 2 shows a schematic of the electron-beam lithography method used to fabricate nanowire sensors attached to gold pads for electrical measurements. The steps involved include electron-beam lithography, development of the exposed pattern, metallization followed by liftoff, reactive ion etching, and finally metal removal to fabricate the device. These procedures are standard methods of fabrication developed in the semiconductor industry and condensed matter physics, and have the potential to be mass produced. All the steps have been carried out in our laboratory in the Physics department and the Photonics Center at Boston University. The sensitivity of the devices arises from the large surface-to-volume ratio at the nanoscale.

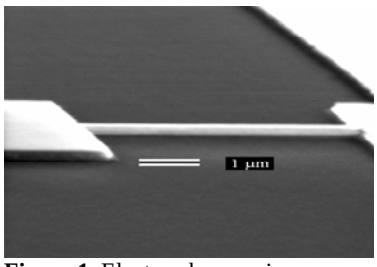

Figure 1. Electron beam microscope image of a single nanowire sensor without control gates.

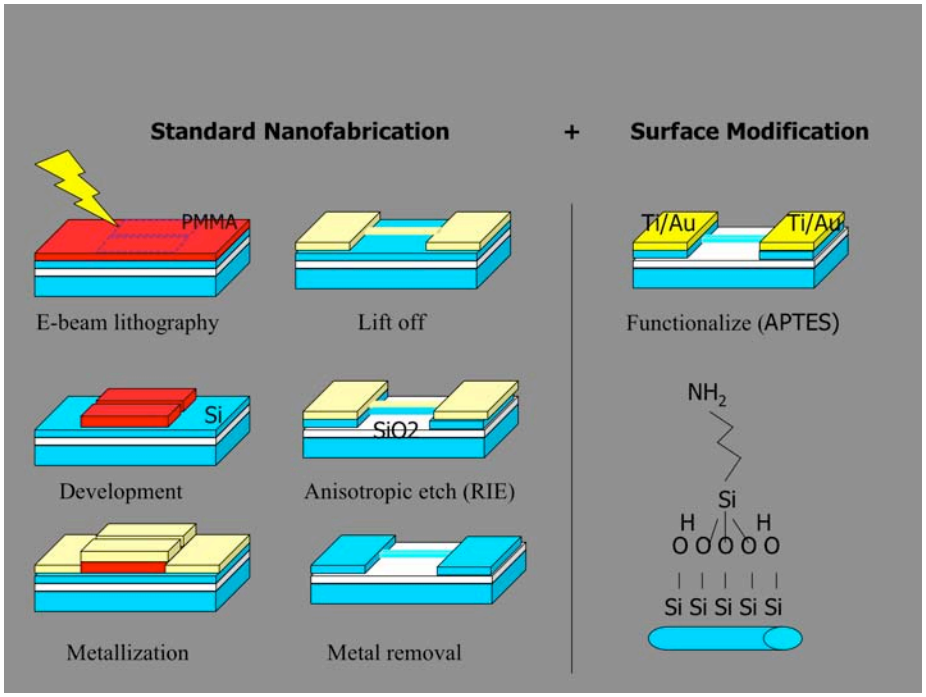

Figure 2. Schematic of the procedures used for device fabrication and surface functionalization of nanowire device.

The surface functionalization protocols are based on silane chemistry, and have the ability to bind to any selected antibody for specificity. Our research work has shown that both sensitivity and specificity to breast cancer biomarkers has been achieved by silicon nanowire sensors.

Figure 3 (next page) shows that we have successfully fabricated an array of nanosensors with controlled dimensions for highly parallel studies on functionalized nanowires. These images demonstrate that lithography methods have the potential for producing special purpose biomarker chips. Unlike fluorescence or optical methods, the chips make measurements purely electrically. The semiconductor industry has long shown that it can make electrical devices smaller, faster and cheaper. In Figure 3(a), an optical micrograph picture also shows the mating to a plastic fluidic channel. The channel has a volume of about 35 μl, and allows for fluid exchange, mixing and sequential testing using a syringe pump. Unlike other microfluidic channel devices, where mixing is a problem, this device allows mixing to occur in the sample volume just above the nanowire. The electrodes connecting to the external electronics are insulated from the serum or buffer. The entire device is mounted onto a semiconductor chip carrier, for easy integration with electronics. Figure 3(b) shows an electron

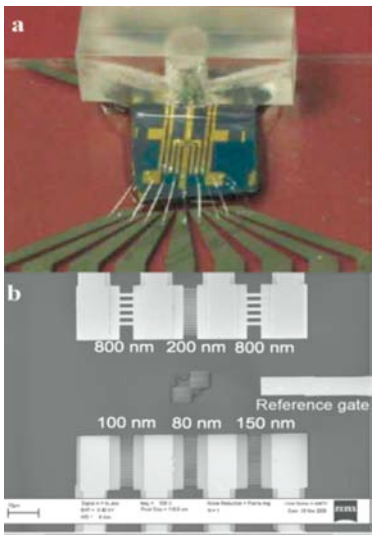

Figure 3. (*Top*) Optical micrograph of the device, showing the flow chamber. (*Bottom*) Electron microscope image of an array of nanosensors of different widths.

microscope image of an array of nanowires, with different geometries for systematic study. Also shown is a reference gate electrode for defining the bias.

Figure 4 shows the device configuration used. An optional top gate is fabricated, and used for calibration studies. The top gate is insulated electrically from the nanowire with an Aluminum oxide layer grown by Atomic Layer Deposition. Parallel arrays of nanowires can be fabricated for signal enhancement. The electon beam writing was performed in the Physics department, and the rest of the fabrication was carried out at the Photonics Center.

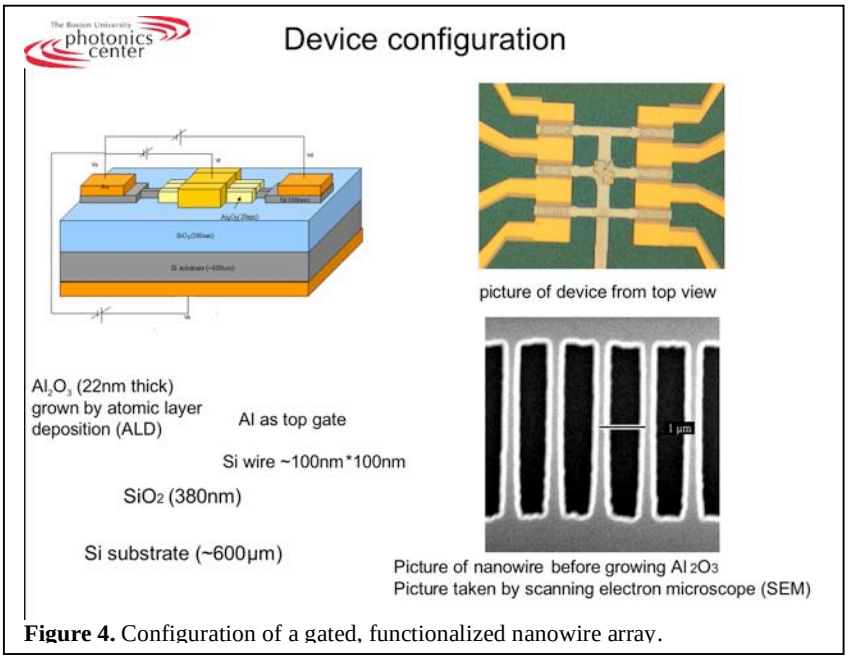

Figure 4. Configuration of a gated, functionalized nanowire array.

Using these devices, we have been able to study, for the first time, what happens to nanowire conductance as the dimension of the nanowire are systematically varied. Other methods that do not use lithography are not able to do this in a systematic way. Our method has complete control of the geometry. Such studies answer the question about 'why nanotechnology'. It can be seen that as the nanowire width is made smaller than about 100 nm, there is dramatic sensitivity to the voltage on the top gate. Binding of the surface markers changes the surface potential and there is also a similar increase in sensitivity to the surface binding. Figure 5 shows the dramatic increase in the sensitivity to the top gate votage as the width of the nanwires is decreased below about 100 nm. Unexpectedly, we found that there was greater sensitivity in the negative V_{ds} regime (drain-source voltage). This novel phenomenon is being investigated for its greater potential sensitivity.

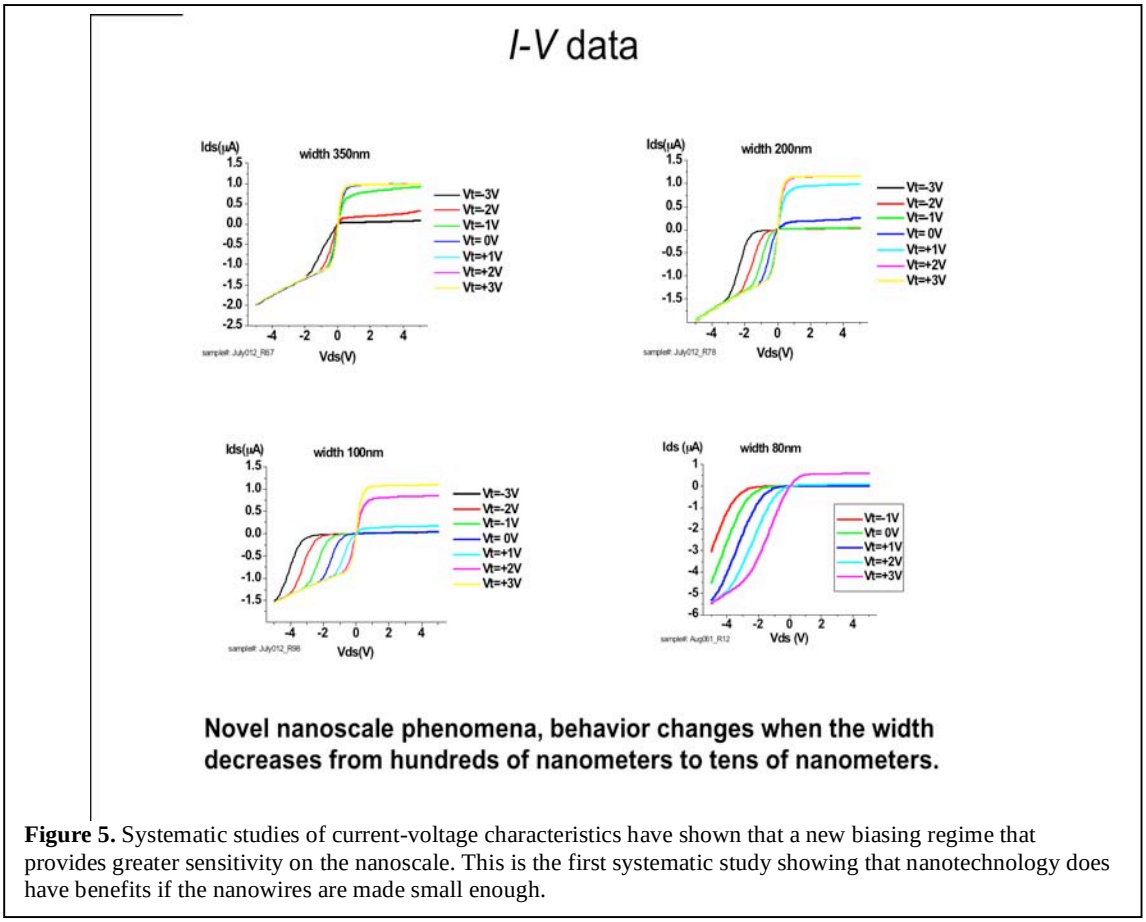

Figure 5. Systematic studies of current-voltage characteristics have shown that a new biasing regime that provides greater sensitivity on the nanoscale. This is the first systematic study showing that nanotechnology does have benefits if the nanowires are made small enough.

Finally, the most important experiments involve the testing of the nanowires against a selected biomarker target. We have shown for the first time that silicon nanowires can detect the presence of the cancer antigen, the mucin related antibody CA15.3. We examined the breast cancer serum biomarker protein CA15.3, a product of the *MUC1* gene. Serum levels of CA15.3 reflect tumor burden, and serial assays of CA15.3 can help assess disease progression and treatment response. The upper limit of normal levels of CA15.3 is ~ 40 Units/milliliter (U/ml). We demonstrate the ability of our nanoscale BIOFET device to detect CA15.3 well below this level. Shown is Figure 6 are the measured changes in conductance as the concentrationof the CA15.3 is varied, under different bias conditions. Figure 6 shows the differential conductance change induced as the antibody functionalized nanowire is challenged by varying concentrations of CA15.3, along with a measurement using antibiotin as control (Fig. 6B). The data indicate that the conductance change observed is primarily due to specific binding of the biomarker. The lower inset in Fig. 6A shows the real-time signal when exposed to a calibrated CA15.3 concentration of 36 U/ml. The device response time is less than 60 seconds. The detection limit of CA15.3 concentration is ~2 U/ml (~10 pM). Furthermore, the BIOFET response as a function of CA15.3 concentration, demonstrated here over a span of 0 U/ml—2,300 U/ml, underlines the wide dynamic range, which can be

applied in a wide range of clinical situations, for both early-stage diagnosis and later-stage prognosis without reconfiguration. Figure 7 shows the level of specificity achieved to date. The signal response to CA15.3 to a model protein shows the specificity. Some residual non-specific binding does exist, but at a level that can be distinguished easily from background.

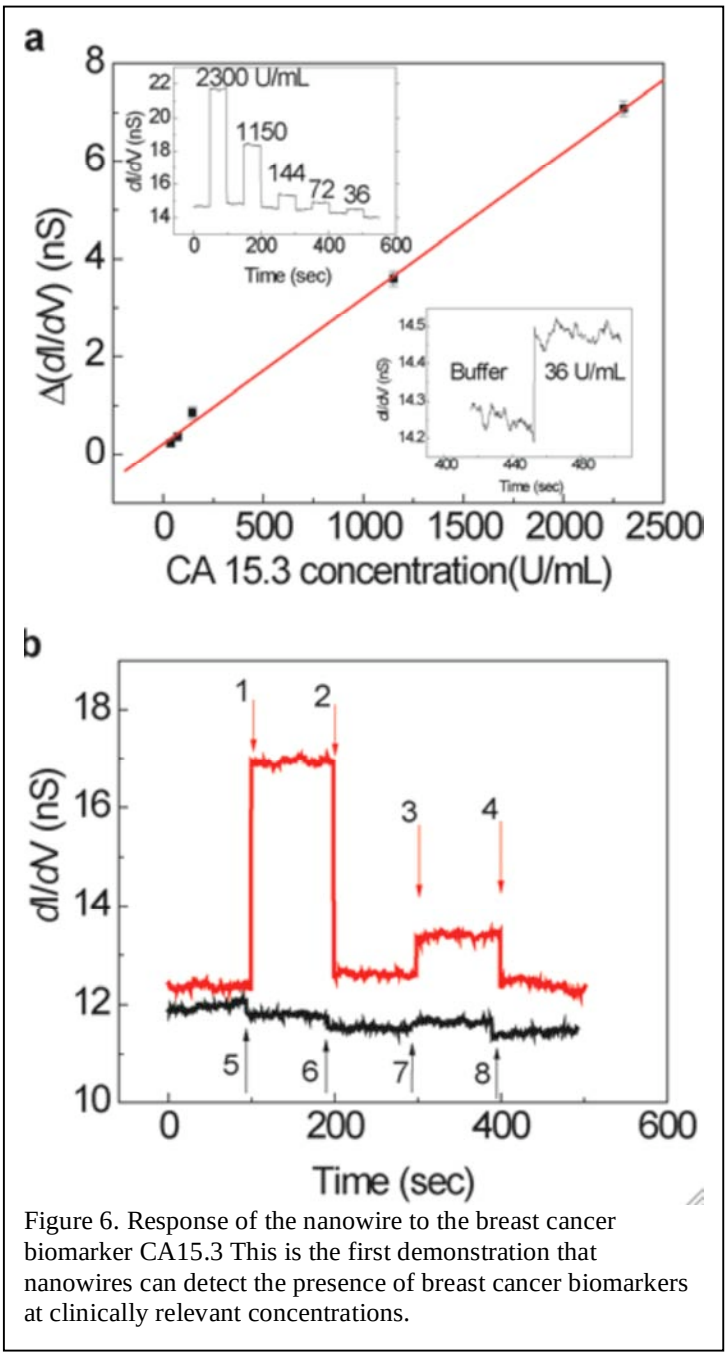

Figure 6. Response of the nanowire to the breast cancer biomarker CA15.3 This is the first demonstration that nanowires can detect the presence of breast cancer biomarkers at clinically relevant concentrations.

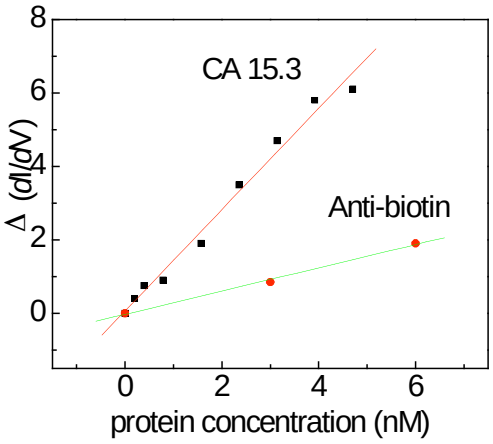

Figure 7. Differential conductance change in the nanowire sensor challenged by CA15.3 and anitbiotin.

Conclusions

To summarize our key research accomplishments, we have shown that the silicon nanowires are sensitive to the breast cancer antigen CA15.3. We have discovered a potentially extremely interesting “Gate Control” method for enhancing signal and also for control of the local pH at the femtoliter level. We have also found a novel biasing regime which enhances the sensitivity to a point where the presence of the biomarker at concentrations well below clinical levels can be readily detected. We are in the process of obtaining data from samples derived from patients and in writing up the exciting results.

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